Plasma free Fatty Acid uptake and oxidation are already diminished in subjects at high risk for developing type 2 diabetes.

Plasma Free Fatty Acid Uptake and Oxidation Are Already Diminished in Subjects at High Risk for Developing Type 2 Diabetes

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The objective of this study was to investigate to what extent disturbances in fatty acid metabolism found in type 2 diabetes are already present in subjects at high risk for developing diabetes (i.e., impaired glucose tolerance [IGT]). Components of fatty acid metabolism were measured in male subjects with IGT during postabsorptive conditions and during 60 min of exercise (50% Vo_{2max}) with the use of the stable isotope tracer [U-^{13}C]palmitate in combination with indirect calorimetry, and those values were compared with previously published findings in male type 2 diabetic and male obese subjects. No differences were found between groups in energy expenditure and in total fat and carbohydrate oxidation. Rate of appearance and rate of disappearance of plasma free fatty acid (FFA) were lower in subjects with IGT and type 2 diabetes compared with obese subjects ($P < 0.05$). Plasma FFA oxidation was lower in subjects with IGT and type 2 diabetes compared with obese subjects at rest and tended to be lower during exercise (rest: 3.7 ± 0.3, 4.4 ± 0.6, and 6.9 ± 1.0 μmol · kg fat-free mass [FFM]$^{-1}$ · min^{-1}, $P < 0.01$; exercise: 15.0 ± 1.7, 14.1 ± 1.9, and 19.6 ± 1.5 μmol · kg FFM^{-1} · min^{-1} for IGT, type 2 diabetic, and obese subjects, respectively, $P = 0.07$). Triglyceride-derived fatty acid oxidation, however, was elevated in subjects with IGT and type 2 diabetes during exercise (3.6 ± 1.4, 1.4 ± 1.4, and −4.0 ± 2.0 μmol · kg FFM^{-1} · min^{-1} for IGT, type 2 diabetic, and obese subjects, respectively; $P < 0.05$). These data demonstrate that male subjects with a prediabetic condition (IGT) have the same defects in fatty acid utilization as subjects with type 2 diabetes, suggesting that these disturbances may play an important role in the progression from IGT to type 2 diabetes. *Diabetes* **50:2548–2554, 2001**

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ARF, acetate recovery factor; FFA, free fatty acid; FFM, fat-free mass; GC-IRMS, gas chromatograph–isotope ratio mass spectrometer; IGT, impaired glucose tolerance; IMTG, intramuscular triglyceride; OGTT, oral glucose tolerance test; R_a, rate of appearance; R_d, rate of disappearance; TTR, tracer-to-tracee ratio; W_{max}, maximal aerobic power output.

Increased circulating levels of plasma free fatty acid (FFA), a common finding in type 2 diabetic subjects, have been identified as an important factor associated with insulin resistance and development of type 2 diabetes, and they seem to be related to common risk factors for coronary heart diseases, including hypertriglyceridemia, hyper–apo B, and increased coagulation activity (1). In the liver, an increased delivery of FFA can impair insulin-mediated suppression of hepatic glucose output (2), increase synthesis of VLDL (1), and diminish hepatic insulin clearance (3). Chronically elevated levels of circulating FFA may also reduce insulin secretion by the β-cell of the pancreas (4,5).

Elevated FFA concentrations may result from increased fasting rates of lipolysis, which seem to result from both the enlarged adipose tissue stores and an impaired insulin-mediated suppression of lipolysis (6). On the other hand, defects in FFA uptake and/or oxidation could also result in elevated circulating levels of FFA. Colberg et al. (7) reported that in viscerally obese women, there was a negative relation between visceral fat content and postabsorptive FFA utilization by skeletal muscle. Similar results were shown by Kelley and associates (8,9) using the leg-balance technique in combination with indirect calorimetry. They reported a diminished uptake of plasma FFA and a diminished oxidation rate of fatty acids in the postabsorptive state in patients with type 2 diabetes in comparison with lean healthy subjects. We have shown in obese type 2 diabetic patients that the uptake and oxidation of plasma FFA are diminished in the forearm muscles in the postabsorptive state and during β-adrenergic stimulation (10). Type 2 diabetic subjects also had a diminished plasma FFA oxidation and a higher triglyceride-derived fatty acid oxidation at the whole-body level as compared with weight-matched control subjects during rest and moderate-intensity exercise (50% Vo_{2max}) (11).

Skeletal muscle is believed to be the main site responsible for the impaired fatty acid utilization and oxidation because of its large mass and since lipids are the principal oxidative substrate of skeletal muscle after an overnight fast and during moderate-intensity exercise. An imbalance between FFA uptake and FFA could easily lead to fat accumulation within skeletal muscle. Indeed, skeletal muscle triglyceride content is increased up to sixfold in patients with diabetes (12,13). Furthermore, the content of intramuscular triglycerides (IMTGs) shows a strong nega-

TABLE 1
Characteristics of the subjects

	Obese (n = 7)	IGT (n = 7)	type 2 DM (n = 7)	ANOVA
Age (years)	45.1 ± 1.7	58.3 ± 2.4*	51.3 ± 3.4	$P < 0.01$
Body weight (kg)	100.8 ± 3.7	93.0 ± 3.3	110.8 ± 7.1	
Body fat (%)	35.0 ± 1.2	33.0 ± 1.2	36.6 ± 1.3	
FFM (kg)	65.6 ± 2.9	62.3 ± 2.3	70.1 ± 4.2	
Waist circumference (cm)	109 ± 2.8	109 ± 1.7	120 ± 3.9	$P < 0.05$
WHR	1.04 ± 0.04	1.04 ± 0.01	1.06 ± 0.03	
Vo_{2max} (ml/min)	2,724 ± 152	2,471 ± 132	2,525 ± 217	
Vo_{2max} (ml/kg FFM/min)	41.5 ± 1.2	39.6 ± 0.9	36.2 ± 2.9	
W_{max} (W)	188 ± 8.5	159 ± 12	184 ± 15	
W_{max} (W/kg FFM)	2.88 ± 0.1	2.54 ± 0.1	2.63 ± 0.2	
Blood glucose (mmol/l)	5.3 ± 0.1	6.2 ± 0.4	7.7 ± 0.5*†	$P < 0.01$
FFA (μmol/l)	727 ± 69	571 ± 93	770 ± 95	

Data are means ± SE. *Significantly different from obese ($P < 0.05$, Sheffe post hoc test); †significantly different from IGT ($P < 0.05$, Sheffe post hoc test). ANOVA, analysis of variance; WHR, waist-to-hip ratio.

tive correlation with the degree of insulin resistance (i.e., insulin-stimulated glucose disposal) (14,15).

Thus, there is a lot of evidence indicating that disturbances in muscle fatty acid utilization may play an important role in the etiology of insulin resistance and type 2 diabetes. However, it is difficult to assess the pathophysiological abnormalities leading to type 2 diabetes when the type 2 diabetic state has already developed, since it is impossible to differentiate between primary factors and adaptational responses. Subjects with impaired glucose tolerance (IGT) have an elevated risk for the development of type 2 diabetes. Within 10 years, up to 50% of individuals with IGT become diabetic (16). If the disturbances in skeletal muscle fatty acid metabolism described above are already present in the glucose-intolerant state, this could indicate that the defects might be important etiological factors in the development of type 2 diabetes.

Therefore, the objective of this study was to investigate to what extent disturbances in fatty acid metabolism found in the diabetic state are already present in subjects at high risk for developing type 2 diabetes (i.e., IGT). We measured components of fatty acid metabolism with the use of stable isotope tracers in combination with indirect calorimetry in subjects with IGT and compared those values with previously published findings in type 2 diabetic subjects and obese subjects during postabsorptive conditions and during 60 min of moderate-intensity exercise (11).

RESEARCH DESIGN AND METHODS

Subjects. Seven obese normoglycemic, seven IGT, and seven type 2 diabetic male subjects were studied. IGT was determined during a 2-h oral glucose tolerance test (OGTT) according to the World Health Organization criteria of 1985. Type 2 diabetes was diagnosed with an OGTT. Diabetic subjects were treated with diet alone (n = 3) or diet together with sulfonylureas (n = 4); blood glucose–lowering medication was withheld for 2 days before the experiment. Subjects had no other health problems besides diabetes/IGT and did not use other medications. Before the start of the study, a medical history and physical examination were performed, and a resting electrocardiogram was also performed. The participants did not perform regular intensive exercise or follow a strict diet or weight-reduction program for the year previous to the start of the study. Subjects were selected from different cohorts and matched as closely as possible for body composition and aerobic capacity. Table 1 lists the subjects' characteristics. The Medical Ethical Review Committee of Maastricht University approved the study protocol, and all subjects gave their written informed consent before the start of the study.

Pretest. Before the start of the experiment, an incremental exhaustive exercise test was performed on an electronically braked bicycle ergometer (Lode, Groningen, the Netherlands) to determine the maximal aerobic power output (W_{max}) and $Vo_{2\,max}$. The test started at a workload of 0.75 W/kg fat-free mass (FFM) for 3 min, followed by 3 min at 1.5 W/kg FFM. Thereafter, the workload was increased every 3 min by 0.5 W/kg FFM until exhaustion (respiratory quotient >1.1 and no further increase in oxygen uptake). Throughout the whole experiment, heart rate, O_2 consumption, and CO_2 production were measured to determine maximal Vo_2 and heart rate. W_{max} was calculated using the time spent on the last workload before exhaustion.

Body composition. Body weight was determined on an electronic scale; body composition was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000; Mijnhardt, Bunnik, the Netherlands) and calculated according to the method of Siri (17). Waist and hip circumference measurements were made with subjects standing in the upright position to the nearest 1 cm, halfway between the spina iliaca and the last rib, and at the level of the trochanter major, respectively.

Study design. Each subject participated in random order in two different trials, separated by at least 1 week. Subjects were asked not to participate in any exhausting physical activity during the 3 days immediately preceding the trials to exclude changes in substrate metabolism induced by the last bout of exercise. They also were asked not to consume any products of high natural ^{13}C-abundance during the week immediately preceding both tests, as this might disturb the $^{13}C/^{12}C$ measurement in blood and expired air (18). In the palmitate test, total fat and carbohydrate oxidation as well as several parameters of fat metabolism were determined by means of the stable isotope technique with a [U-^{13}C]palmitate tracer (Protocol 1) in the postabsorptive state while subjects were resting supine on a comfortable bed in a room kept at 21–23°C, and during submaximal exercise (50% Vo_{2max}). During a separate test, the stable isotope tracer [1,2-^{13}C]acetate was infused to determine the acetate recovery factor (ARF) necessary for correction of palmitate oxidation rates for loss of label in the tricarboxylic acid cycle and bicarbonate pool (protocol 2) (19).

Protocol 1. Subjects reported to the laboratory at 8:00 A.M., arriving there by car or bus after a fast that started at 10:00 P.M. the day before. Two cannulas were inserted: one into a forearm vein for the infusion of palmitate, and the other in retrograde direction into a contralateral dorsal hand vein for blood sampling. The cannulated hand was placed in a hot box in which air was circulated at 60°C to arterialize the venous blood. Background blood and breath samples were taken 30 min after placement of the catheters. Then, at t = 0 min an intravenous dose of 0.085 mg/kg $NaH^{13}CO_3$ was given to prime the bicarbonate pool, followed by a constant-rate continuous infusion of [U-^{13}C]palmitate (0.0067 μmol · kg_{bm}^{-1} · min^{-1}) via a calibrated infusion pump (IVAC560 pump; IVAC, San Diego, CA) until the end of the resting period (0–120 min). During the last 20 min of the resting period (t = 100, 110, and 120 min), breath and blood samples were taken. Thereafter, exercise was started at 50% of the predetermined individual Vo_{2max} (~45% W_{max}) for 1 h (120–180 min). The rate of infusion of [U-^{13}C]palmitate was doubled at the start of exercise. During the last 20 min of exercise, blood and breath samples were taken (t = 160, 170, and 180 min). The palmitate tracer (potassium salt of [U-^{13}C]palmitate, 99% enriched; Cambridge Isotope Laboratories, Andover, MA) was dissolved in heated sterile water and passed through a 0.2-μm filter

into 5% warm human serum albumin (Central Blood Bank, Leiden, the Netherlands) to make a 0.65 mmol/l solution. The exact infusion rate of [U-^{13}C]palmitate was determined for each experiment by measuring the concentration of the infusate.

Protocol 2. During this trial, the same regimen as described for protocol 1 was followed, with the exception that in place of a [U-^{13}C]palmitate tracer, a [1,2-^{13}C]acetate tracer was infused. A priming dose of $NaH^{13}CO_3$ (0.085 mg/kg) was given, followed by a constant-rate continuous infusion of [1,2-^{13}C]acetate at 0.046 μmol · kg_{bm}^{-1} · min^{-1} during rest (0–120 min) and twice during exercise (120–180 min), thereby giving the same ^{13}C infusion rate as for palmitate. At the same time points as described in protocol 1, breath samples were obtained, and no blood samples were drawn. The acetate tracer (sodium salt of [1,2-^{13}C]acetate, 99% enriched; Cambridge Isotope Laboratories) was dissolved in 0.9% saline and infused at the same ^{13}C infusion rate per time unit as that for the palmitate tracer.

Breath, blood, and urine sampling. Breath samples were obtained by having the subjects breathe normally for at least 3 min into a mouthpiece connected to a 6.75-liter mixing chamber and then collecting a breath sample into a 20-ml vacutainer tube (Becton Dickinson, Meyland Cedex, France) to determine the enrichment of CO_2 ($^{13}C/^{12}C$ ratio). Vo_2 and Vco_2 were determined by means of open-circuit spirometry (Oxycon β; Mijnhardt) during the resting period and the last 30 min of exercise. Arterialized blood samples were collected in EDTA-containing tubes on ice and were immediately centrifuged at 3,000 rpm at 4°C, and the plasma was put away in liquid nitrogen and stored at −80°C until analysis. Urine was collected overnight in containers with H_2SO_4 to determine nitrogen excretion for calculating the nonprotein respiratory exchange ratio.

Biochemical methods. Breath samples were analyzed for $^{13}C/^{12}C$ ratio by injecting 20 μl of the gaseous head space into a gas chromatograph–isotope ratio mass spectrometer (GC-IRMS) (Finnigan MAT 252; Finnigan, Bremen, Germany). Total plasma FFA, glucose, and infusate acetate concentrations were measured using standard enzymatic techniques automated on the Cobas Fara centrifugal analyzer at 340 nm (for FFA: FFA-C test kit; Wako Chemicals, Neuss, Germany; for glucose: Roche Unikit III; Hoffman-LaRoche, Basel, Switzerland; for acetate: kit no. 148261; Boehringer Mannheim, Mannheim, Germany). For the determination of plasma palmitate concentration and enrichment, FFAs were extracted from plasma, isolated by thin-layer chromatography, and derivatized to their methyl esters. Palmitate concentrations were determined on an analytical gas chromatograph with ion-flame detection using heptadecanoic acid as an internal standard; on average, palmitate concentration was 25 ± 1% of total FFA concentration. Isotopic enrichment of palmitate was determined by GC-IRMS after online combustion of fatty acids to CO_2 (Finnigan MAT 252), with correction for the extra methyl group in the derivate. The concentration of infusate palmitate was determined as described above for plasma samples.

Calculations. The metabolic rate was calculated from Vo_2 and Vco_2 according to the equation of Weir (20). Carbohydrate and fat oxidation rates were calculated from Vo_2, Vco_2, and urinary nitrogen excretion (21). Protein oxidation (as calculated from nitrogen excretion) was assumed to be similar during the overnight-fasted state and during exercise.

Tracer calculations. Total fatty acid oxidation was calculated by converting the rate of fat oxidation (triglyceride oxidation) to its molecular equivalent, with the assumption that the average molecular weight of triglyceride was 860 g/mol, and multiplying the molar rate of triglyceride oxidation by 3 because each molecule contains 3 mol/l of fatty acids.

Enrichment of breath CO_2 and plasma palmitate and acetate is given as tracer-to-tracee ratio (TTR).

$$\text{TTR} = (^{13}C/^{12}C)\ \text{sample} - (^{13}C/^{12}C)\ \text{background}.$$

Fractional recovery of label in breath CO_2, derived from the infusion of labeled acetate, is calculated as follows:

$$\text{acetate recovery} = (\text{TTR}co_2 \times Vco_2)/2F$$

where F is the infusion rate of acetate (millimoles per minute) and the number 2 in the denominator is to correct for the number of ^{13}C molecules in acetate. Vco_2 is the expired CO_2 (millimoles per minute).

During the last 20 min of the resting period (time points 110 and 120 min), a physiological and isotopic steady state was present; therefore, Steele's equation for steady state was applied to calculate the palmitate rate of appearance (R_a) or rate of disappearance (R_d).

$$\text{palmitate } R_a/R_d\ (\mu\text{mol/min}) = (F/\text{TTR plasma palmitate}) - F$$

where F is the infusion rate of palmitate (micromoles per minute).

During the last 20 min of the exercise period (160–170 and 170–180 min), the single-pool non–steady state equations of Steele adapted for use with stable isotopes were used to calculate R_a and R_d of palmitate.

$$\text{Total } R_a \text{ palmitate} = \frac{F - V[C_2 + C_1/2]\,[(\text{TTR}_2 - \text{TTR}_1)/(t_2 - t_1)]}{(\text{TTR}_2 + \text{TTR}_1)/2}$$

$$\text{Total } R_d \text{ palmitate} = R_a - V(C_2 - C_1)/(t_1 - t_2)$$

where F is the infusion rate (μmol · kg^{-1} · min^{-1}); V is the volume of distribution, which was assumed to be 0.040 l/kg; C_1 and C_2 are the palmitate concentrations at times t_1 and t_2, respectively.

The R_a and R_d of FFA during rest and exercise, respectively, were calculated by dividing the palmitate flux and palmitate R_a and R_d by the fractional contribution of palmitate to the total FFA concentration.

The percent of infused [U-^{13}C]palmitate oxidized was calculated with the following formula:

$$\%\ \text{infused tracer oxidized} = \frac{(\text{TTR}co_2 \times Vco_2)}{(16 \times F \times \text{acetate recovery})} \times 100\%$$

where F is the infusion rate of palmitate (micromoles per minute) and the number 16 in the denominator is to correct for the number of ^{13}C molecules in palmitate. Vco_2 is the expired CO_2 (micromoles per minute).

At the end, plasma FFA oxidation (micromoles per minute) was calculated as follows:

$$\text{plasma FFA oxidation} = R_d\text{FFA} \times \%\ \text{infused palmitate tracer oxidized}$$

The fatty acid oxidation from plasma triglycerides and IMTGs was calculated according to the following formula:

$$\text{triglyceride-derived FA oxidation} = \text{total FA oxidation} - \text{plasma FFA oxidation}$$

Statistical analysis. Data are presented as means ± SE. Oxidation rates and R_a and R_d are expressed as μmol · kg FFM^{-1} · min^{-1}. Differences among groups were analyzed by a two-way analysis of variance. Changes in concentration of metabolites over time were analyzed with a repeated-measures analysis of variance. Statistical significance was set at $P < 0.05$. In case of significance, Scheffe's post hoc test was performed.

RESULTS

Subject characteristics and exercise intensity. Subjects were matched for body composition and aerobic capacity; there were no significant differences in percentage body fat, FFM, and W_{max} (Table 1). Type 2 diabetic subjects had a larger waist circumference compared with IGT and obese subjects; waist-to-hip ratio, however, was not different. Subjects with IGT tended to have a lower body weight ($P = 0.06$); also, they were older than the obese subjects ($P < 0.01$). Fasting blood glucose level was 7.7 ± 0.5 mmol/l in type 2 diabetic subjects, which was significantly elevated in comparison with obese and IGT subjects ($P < 0.01$).

During the last 20 min of exercise, oxygen consumption reached a plateau (data not shown). No differences were observed in oxygen consumption among groups during exercise (1.55 ± 0.07, 1.36 ± 0.06, and 1.35 ± 0.11 l/min for obesity, IGT, and type 2 diabetes). During the last 20 min of exercise, mean relative workload was comparable (57.8 ± 3.6, 55.2 ± 2.1, and 54.9 ± 4.6% Vo_{2max} for obesity, IGT, and type 2 diabetes).

Arterialized concentrations of metabolites. Plasma glucose levels were higher during rest in type 2 diabetic subjects than in obese and IGT subjects ($P < 0.05$). During exercise, blood glucose levels declined in type 2 diabetic subjects ($P < 0.05$) and remained stable in obese and IGT subjects; differences among groups in blood glucose concentration were no longer significant during exercise. Insulin levels were elevated in type 2 diabetic subjects at rest and during exercise ($P < 0.05$). Insulin levels declined as a result of the exercise ($P < 0.01$); the decline was not different among groups. Circulating levels of plasma FFA were not different among groups at rest, nor were they

TABLE 2
Plasma glucose, insulin, and FFA levels of obese, IGT, and type 2 diabetic subjects during rest and exercise

	Rest	Exercise			Repeated-measures ANOVA	
		160 min	170 min	180 min	Exercise	Group × exercise
Glucose (mmol/l)						
Obese	5.2 ± 0.2*	5.3 ± 0.3	5.4 ± 0.3	5.5 ± 0.3		
IGT	5.6 ± 0.4*	5.2 ± 0.3	5.2 ± 0.3	5.1 ± 0.3	$P < 0.01$	$P < 0.001$
Type 2 diabetes	6.7 ± 0.4	6.1 ± 0.3	6.0 ± 0.5	5.9 ± 0.5		
Insulin (mU/l)						
Obese	10.5 ± 0.6*	7.7 ± 0.6*	8.7 ± 0.8*	8.1 ± 0.4*		
IGT	9.1 ± 1.7*	6.8 ± 1.1*	6.9 ± 1.4*	6.5 ± 1.5*	$P < 0.0001$	NS
Type 2 diabetes	19.0 ± 2.5	13.3 ± 2.1	13.1 ± 2.2	13.3 ± 2.0		
FFA (μmol/l)						
Obese	727 ± 70	728 ± 60	836 ± 62	861 ± 84		
IGT	562 ± 109	599 ± 111	670 ± 106	730 ± 107	$P < 0.0001$	NS
Type 2 diabetes	694 ± 65	608 ± 107	747 ± 138	816 ± 148		

Data are means ± SE, unless otherwise stated. *Significantly different from type 2 diabetes ($P < 0.05$, Sheffe post hoc test). ANOVA, analysis of variance.

different during exercise (Table 2). Plasma FFA levels increased during the exercise period ($P < 0.01$) and kept increasing throughout the whole exercise period in all three groups to the same extent.

Substrate utilization and energy expenditure. Whole-body energy expenditure and total fat and carbohydrate oxidation reached a plateau during the last 20 min of exercise (data not shown). Energy expenditure was comparable in all three groups during resting conditions and during exercise (rest: 6.45 ± 0.34, 5.23 ± 0.17, and 5.65 ± 0.49 kJ/min; exercise: 31.8 ± 1.5, 27.8 ± 1.4, and 27.7 ± 2.3 kJ/min for obesity, IGT, and type 2 diabetes, respectively). During exercise, energy expenditure increased four- to fivefold. Respiratory exchange ratio was also not significantly different among groups during rest and exercise (rest: 0.79 ± 0.01, 0.83 ± 0.01, and 0.80 ± 0.02; exercise: 0.88 ± 0.02, 0.85 ± 0.01, and 0.87 ± 0.01 for obesity, IGT, and type 2 diabetes, respectively). No significant differences were observed among groups in total carbohydrate oxidation (rest: 1.28 ± 0.28, 1.94 ± 0.28, and 1.45 ± 0.30; exercise: 18.3 ± 2.04, 14.1 ± 1.38, and 13.8 ± 1.63 mg · kg FFM^{-1} · min^{-1} for obesity, IGT, and type 2 diabetes, respectively) and fat oxidation (rest: 1.40 ± 0.15, 0.91 ± 0.11, and 1.20 ± 0.18; exercise: 4.47 ± 0.59, 5.24 ± 0.57, and 4.56 ± 0.60 mg · kg FFM^{-1} · min^{-1} for obesity, IGT, and type 2 diabetes, respectively) as measured with indirect calorimetry.

Plasma palmitate ^{13}C enrichment was slightly elevated in subjects with IGT compared with obese and type 2 diabetic subjects at rest ($P < 0.05$); however, during the exercise period, these differences were no longer significant. Acetate recovery gradually increased during both rest and exercise (Fig. 1). No differences were found in ARF among groups under resting conditions; during exercise, ARF tended to be higher in obese subjects compared with subjects with IGT and type 2 diabetes ($P = 0.07$) (Fig. 1).

R_a and R_d of plasma FFA were significantly different ($P < 0.05$) among groups under baseline and exercise conditions. Both R_a and R_d were highest in obese subjects and comparable in subjects with IGT and type 2 diabetes (Fig. 2).

Baseline and exercise-induced total fatty acid oxidation were not different among the groups (Fig. 3*A*). Plasma FFA oxidation was decreased in subjects with IGT and type 2 diabetes compared with obese subjects during rest ($P < 0.05$) (Fig. 3*B*). During exercise, there was a tendency toward a lower plasma FFA oxidation in subjects with IGT and type 2 diabetes ($P = 0.07$) (Fig. 3). Triglyceride-derived fatty acid oxidation was not different during rest ($P = 0.17$) (Fig. 3*C*); during exercise, however, triglyceride-derived fatty acid oxidation was elevated in subjects with IGT and type 2 diabetes in comparison with obese subjects ($P < 0.05$) (Fig. 3).

The percentage of the R_d that was oxidized during rest and exercise was not significantly different among groups (rest: 40.0 ± 4.0, 35.6 ± 2.0, and 34.1 ± 2.5% for obesity, IGT, and type 2 diabetes, respectively, $P = 0.28$; exercise: 72.1 ± 1.5, 76.8 ± 5.1, and 70.8 ± 3.9% for obesity, IGT, and type 2 diabetes, respectively, $P = 0.52$).

DISCUSSION

Disturbances in fatty acid utilization and oxidation may play an important role in the development of obesity and insulin resistance. Type 2 diabetic subjects have a reduced

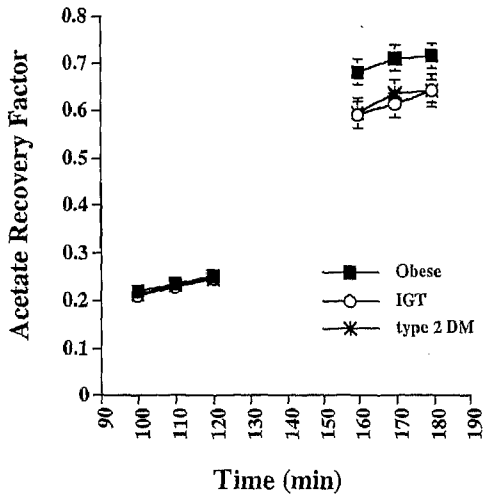

FIG. 1. ARF in obese (■), IGT (○), and type 2 diabetic (*) subjects during rest (100–120 min) and moderate-intensity exercise (160–180 min) (mean ± SE).

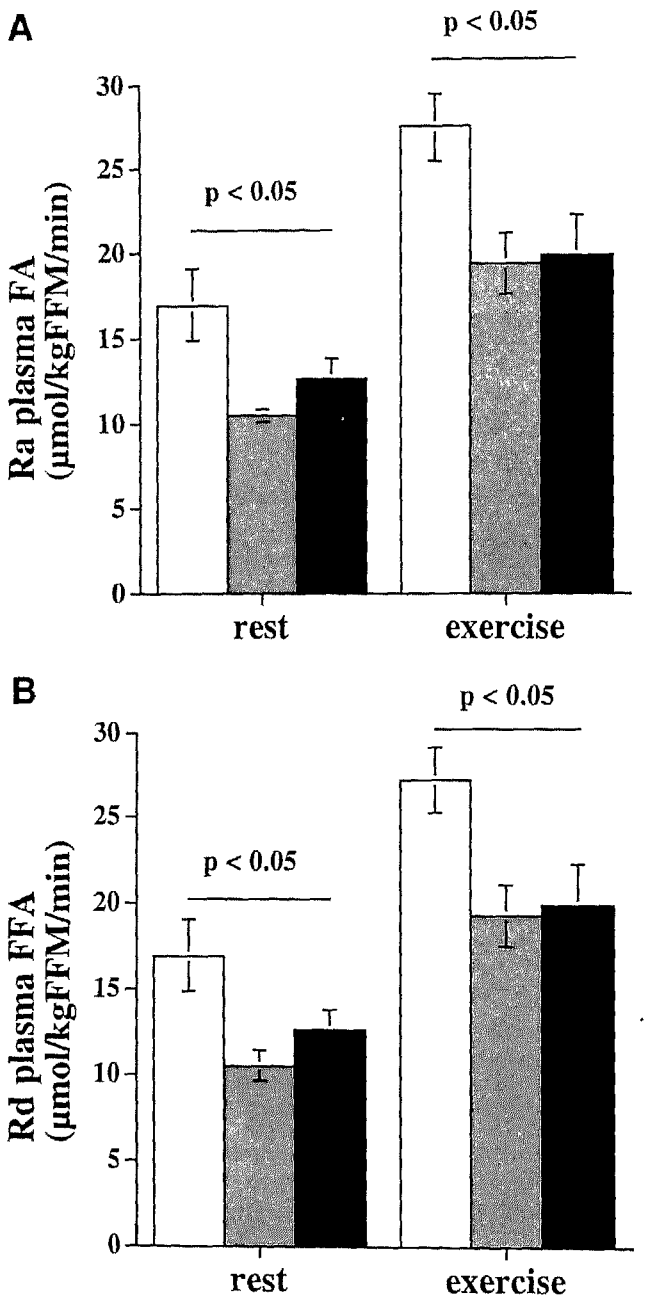

FIG. 2. R_a (*A*) and R_d (*B*) of plasma FFA in obese (□), IGT (▩), and type 2 diabetic (■) subjects during rest and moderate-intensity exercise (mean ± SE). $P < 0.05$ for all.

uptake and oxidation of plasma FFA by muscle during fasting conditions and during β-adrenergic stimulation (8,10). Recently, these findings were extended to moderate-intensity exercise (11). So far, no information is available about whether these disturbances are primary factors or adaptational responses to the diabetic state. The results of the present study showed for the first time that in subjects at high risk for the development of type 2 diabetes, i.e., subjects with IGT, disturbances in fatty acid uptake and oxidation are present. During baseline conditions, plasma FFA oxidation was impaired in subjects with IGT and type 2 diabetes compared with body composition–matched control subjects. During moderate-intensity exercise, plasma FFA oxidation tended to be lower in subjects with IGT and type 2 diabetes than in obese control subjects ($P = 0.07$); however, triglyceride-derived fatty acid oxidation was higher in glucose-intolerant and type 2 diabetic men than in obese men. Thus, impairments in fatty acid oxidation are already present in a prediabetic state (i.e., IGT), suggesting that these disturbances possibly play a role in the progression from IGT to type 2 diabetes.

ARF and isotopic enrichment. Oxidation rates of plasma fatty acid were determined with use of the stable isotope [U-^{13}C]palmitate in combination with indirect calorimetry. To correct for the loss of ^{13}C label due to fixation in products of the tricarboxylic acid cycle and the bicarbonate pool, Sidossis et al. (19) introduced the ARF. Failure to use the ARF may lead to a substantial underestimation of plasma FFA oxidation. A recent study pointed out that the ARF needs to be determined in every subject because of the large interindividual variation, especially during exercise (22). In the present study, the ARF tended to be lower in the glucose-intolerant and diabetic state during exercise compared with obese control subjects ($P = 0.07$). This is in agreement with previous studies showing a lower ARF in type 2 diabetic patients (11,22). The explanation for this is not fully understood, but it is suggested that an increased rate of gluconeogenesis in the IGT or diabetic state leads to additional loss of ^{13}C label from the tricarboxylic acid cycle and thus a lower ARF. This study again stresses the importance of individual determination of the ARF to improve estimations of plasma substrate oxidation.

Subjects. Obese, glucose-intolerant, and type 2 diabetic subjects were matched according to body composition and aerobic capacity. However, subjects with IGT were older than obese and type 2 diabetic subjects (58.3 ± 2.4 years for subjects with IGT vs. 45.1 ± 1.7 and 51.3 ± 3.4 years for obese and type 2 diabetic subjects, respectively; $P < 0.01$). Aging is associated with a diminished ability to use fat as a fuel during exercise (23,24); however, those observations were made comparing young adults (20–33 years of age) with middle-aged (50–55 years of age) or elderly (66–79 years of age) subjects. Furthermore, disturbances in fat utilization in the elderly are partly related to a decline in physical activity observed with aging (24). In this study, no differences in aerobic capacity (Vo_{2max}) were present among the groups. Also, during the exercise period, no significant differences in absolute and relative workload, oxygen consumption, and energy expenditure existed. This finding seems to suggest that the muscle oxidative capacity (mitochondrial density) was matched, too. Thus, on the basis of the comparable Vo_{2max} and the relatively small age differences, it seems highly unlikely that this age difference has disturbed the results of the present study.

Fatty acid metabolism. Total fat and carbohydrate oxidation were not different among the groups throughout the experiment. This result is in agreement with other studies showing that whole-body fat and carbohydrate oxidation were not different in diabetic subjects compared with obese and lean subjects during rest and exercise (25,26). As indicated above, plasma fatty acid uptake and oxidation are lowered, and triglyceride-derived fatty acid oxidation (IMTG or VLDL triglyceride oxidation) is elevated, in subjects with IGT and diabetes compared with obese

control subjects. Thus, although whole-body fat oxidation is the same, there is a redistribution of the source of fatty acids oxidized. Skeletal muscle is by far the most important tissue responsible for uptake and oxidation of lipids, especially during exercise. Increased levels of muscle triglycerides, as reported in diabetic subjects (12,13) and most likely also in subjects with IGT, could lead to increased rates of triglyceride-derived fatty acid oxidation in muscle by a mass action effect, as utilization of muscle triglycerides during exercise is directly related to the content at rest in diabetic subjects (13). This would explain the higher rate of triglyceride-derived fatty acid oxidation in glucose-intolerant and diabetic subjects found in this study. It can be speculated that the tendency toward increased triglyceride-derived fatty acid oxidation may be one of the mechanisms linking increased IMTG content toward insulin resistance, as discussed in more detail below.

In the present study, R_a of plasma FFA was lower in subjects with IGT and type 2 diabetes than in weight-matched obese subjects. Type 2 diabetes is frequently accompanied by obesity, which makes it difficult to ascertain the impact of diabetes itself on lipolysis and FFA release. For that reason, diabetic subjects must be compared with body composition–matched control subjects, as done in this study. Using microdialysis, Jansson et al. (27) concluded that adipose tissue lipolysis (expressed per unit of fat mass) was not different between well-controlled type 2 diabetic subjects and weight-matched control subjects. The lower R_a FFA found in the present study can most likely be explained by a difference in the fraction of FFA undergoing reesterification in adipose tissue and not by differences in adipose tissue lipolysis.

IGT is considered a prediabetic state, a necessary transition state en route to type 2 diabetes. Up to 15% of the people diagnosed with IGT develop diabetes each year (16). It would be of use if subjects at risk for progression to type 2 diabetes could be identified. Major risk factors for progression from IGT to type 2 diabetes are basal and 2-h glucose level, basal proinsulin level, age, and obesity (16). The results of the present study suggest that an impaired skeletal muscle fatty acid metabolism could be another important factor in the progression from IGT to type 2 diabetes. An increased level of physical activity in combination with a healthy diet and weight loss improved glucose tolerance in a Finnish study (28). One could speculate that changes in fatty acid metabolism as a consequence of the change in lifestyle partly explain the improved glucose tolerance.

Underlying mechanisms. Several mechanisms may explain the observed impaired plasma FFA uptake and oxidation in subjects with IGT and type 2 diabetes. Throughout the experiment, the R_a and R_d of plasma FFA were lower in subjects with IGT and type 2 diabetes than in obese subjects ($P < 0.05$). This diminished R_a of plasma FFA could lead to a diminished uptake and subsequent oxidation of plasma FFA. The lower R_a of FFA may possibly be explained by an increased reesterification of fatty acid in adipose tissue or by differences in the regulation of lipolysis between obese subjects and obesity-associated type 2 diabetic subjects (26). However, the present study cannot be conclusive about this possibility. The fraction of the R_d oxidized was not different among groups, which

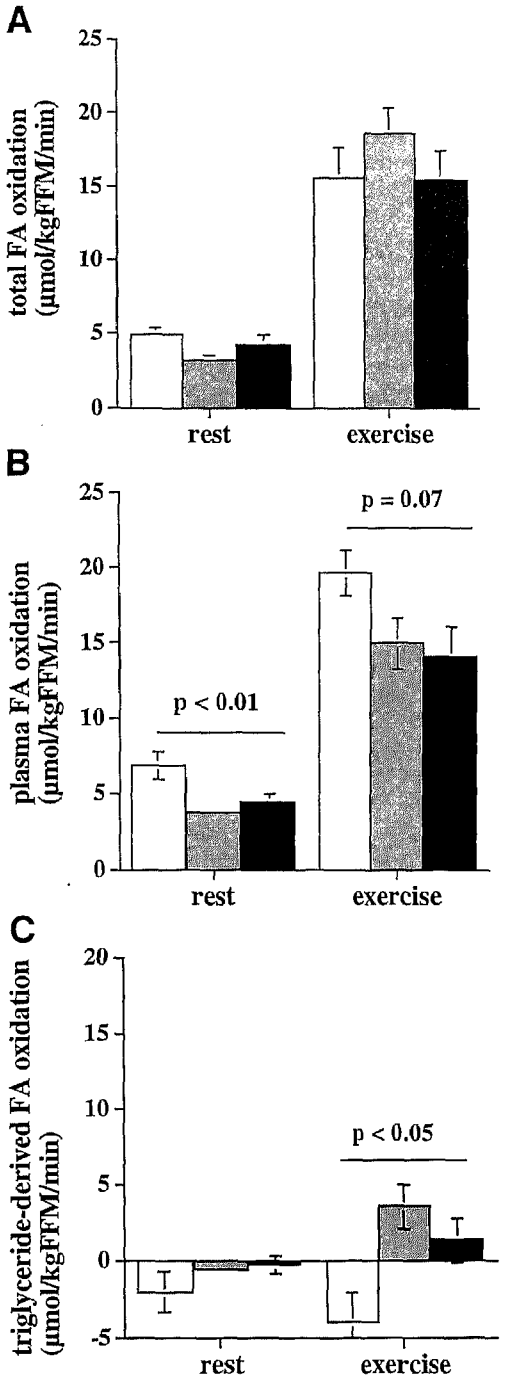

FIG. 3. Total fatty acid oxidation (*A*), plasma fatty acid oxidation (*B*), and triglyceride-derived fatty acid oxidation (*C*) in obese (□), IGT (■), and type 2 diabetic (■) subjects during rest and moderate-intensity exercise (mean ± SE).

means that once taken up, plasma FFAs are oxidized to the same extent in all groups, suggesting that the uptake of plasma FFA is more impaired than its oxidation.

A second factor that may play a role in the disturbed uptake and oxidation of plasma FFA is a lowered transport of FFA across the membrane. Alterations in content or expression of transport proteins could influence the capacity of the muscle to utilize lipids. In muscle biopsies from type 2 diabetic patients, the content of cytoplasmatic fatty acid binding protein was decreased as compared with control subjects, which could contribute to the decreased

utilization and oxidation of plasma FFA (10). More studies, however, are needed to better understand the role and the importance of fatty acid transporters in the disturbances in muscle lipid metabolism as found in the diabetic state.

Thirdly, subjects with IGT may have increased stores of IMTG, as seen in diabetic subjects (12). Enlarged stores of triglycerides within the muscle in combination with an increased rate of lipolysis, as a result of a mass action effect, can flood the prediabetic muscle with FFA and lead to an increased concentration of FFA in the muscle cell. Indeed, we recently observed an increased release of glycerol from the forearm muscle of type 2 diabetic subjects, suggesting an increased lipolysis of IMTGs (10). Elevated levels of fatty acids within the cytosol of the muscle cell would diminish the blood tissue FFA concentration gradient, which is a very important determinant of plasma fatty acid uptake and oxidation and may explain the impaired uptake and oxidation of plasma fatty acid found in subjects with IGT and type 2 diabetes. This hypothesis is a very attractive one, because elevated levels of FFA in the cell could also interfere with insulin signaling and disturb insulin-stimulated glucose uptake (29). Interestingly, the tendency toward increased triglyceride-derived fatty acid oxidation found in this study may link increased IMTG content and utilization to decreased insulin-stimulated glucose uptake (insulin resistance). More studies, however, are needed to draw more definitive conclusions.

In conclusion, this study shows that the previously reported disturbances in fatty acid utilization in type 2 diabetes (8,11) are already present in a prediabetic state, i.e., in subjects with IGT. During rest and exercise, plasma FFA uptake and oxidation were diminished in subjects with IGT and type 2 diabetes, whereas triglyceride-derived fatty acid oxidation was increased during exercise in subjects with IGT and type 2 diabetes compared with obese subjects. An increased IMTG content, a diminished FFA transport capacity, or a decreased R_a of plasma FFA could be involved in the impaired plasma FFA uptake and oxidation. These data demonstrate that male subjects with milder forms of hyperglycemia (IGT) have the same defects in fatty acid utilization as subjects with type 2 diabetes, suggesting that these disturbances may play an important role in the progression from IGT to type 2 diabetes.

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